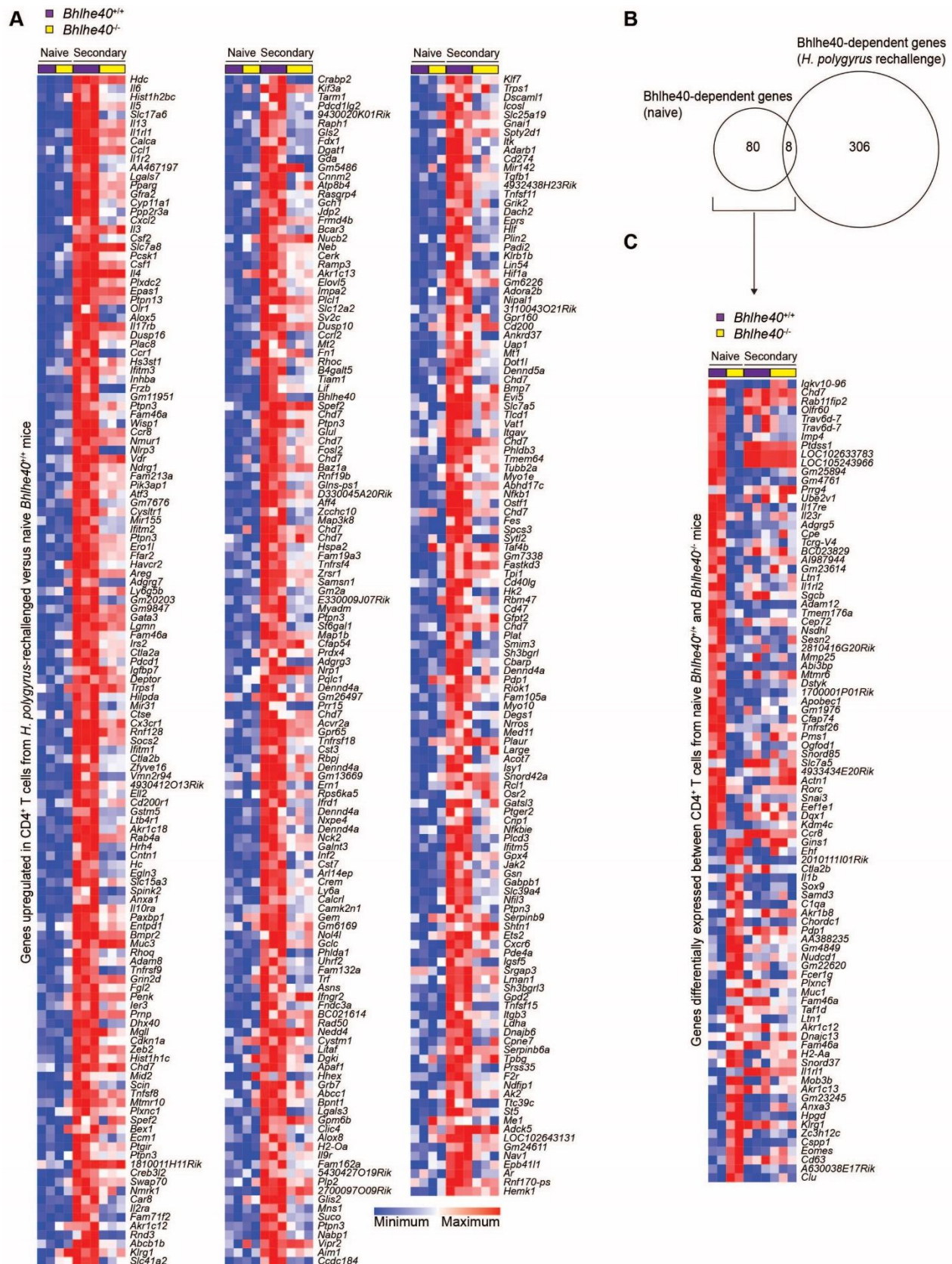
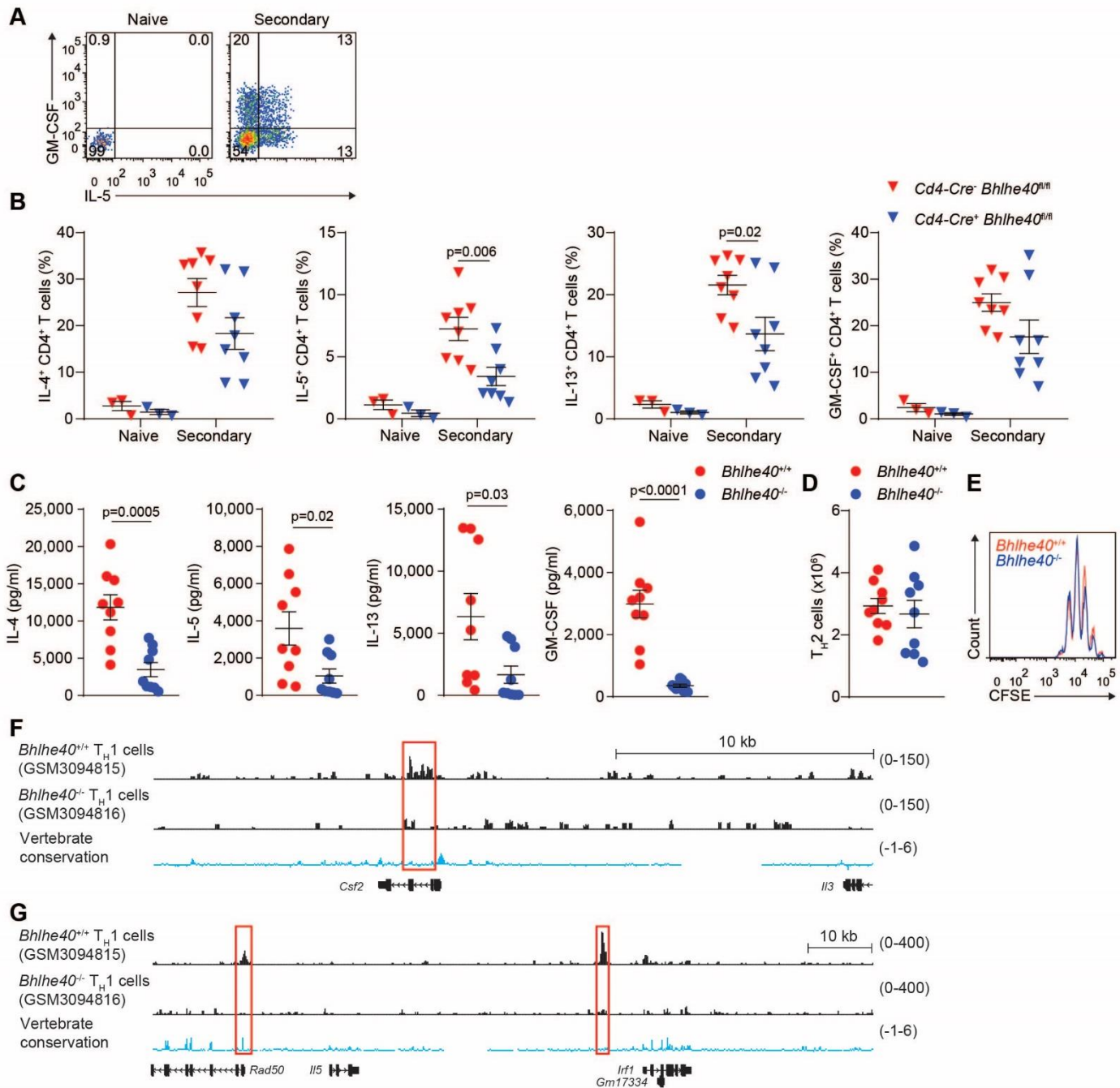


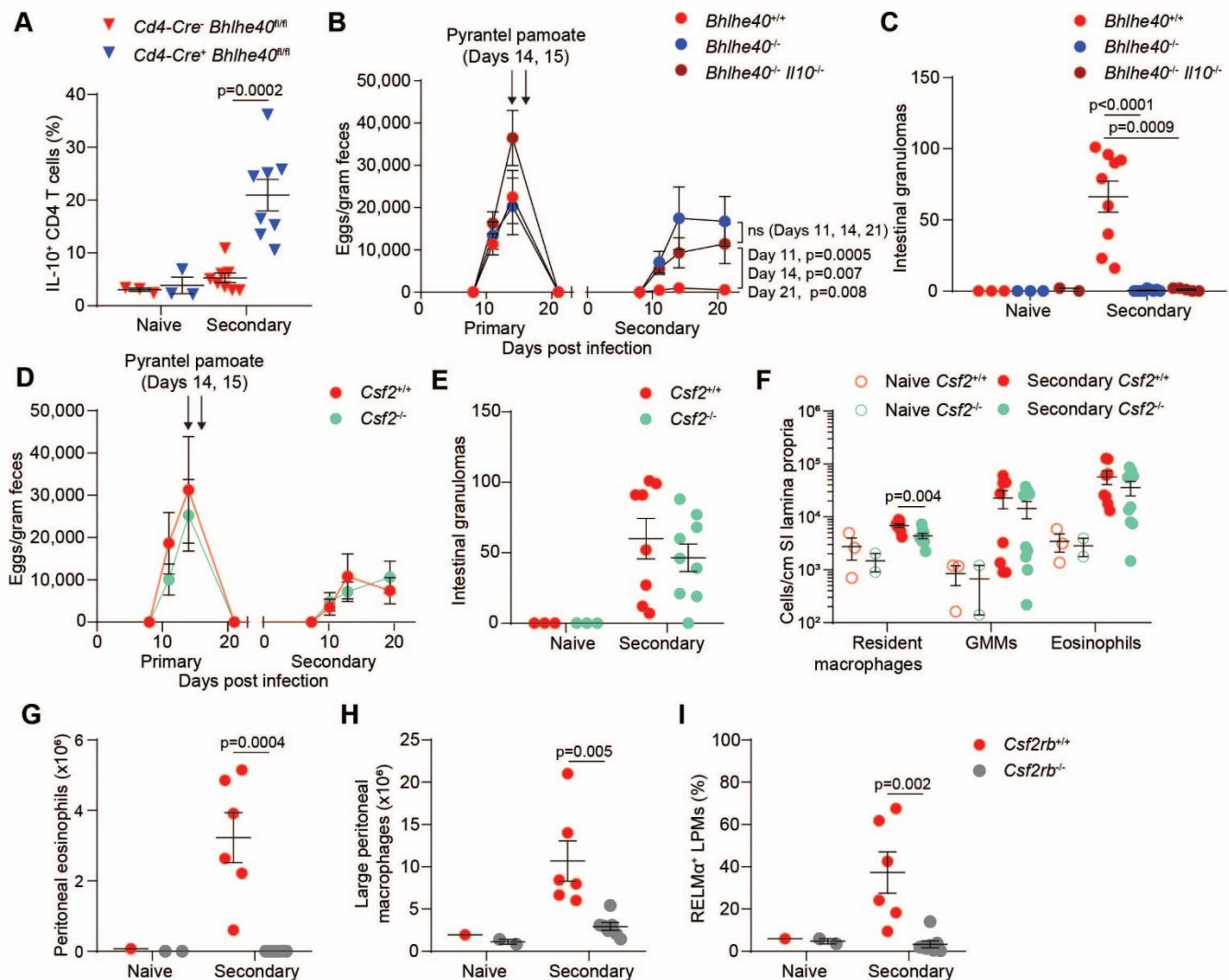
Supplemental Figure 1. Loss of BHLHE40 dysregulates myeloid cell responses to *H. polygyrus* rechallenge. (A) *H. polygyrus*-infected *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice were analyzed for quantitation of adult worms recovered from the intestines of mice experiencing primary or secondary infection. (B and C) Naïve and *H. polygyrus*-rechallenged *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice were analyzed by flow cytometry for (B) SILP eosinophils and (C) quantitation of SILP CD3⁺ T cells. (D-H) Naïve and *H. polygyrus*-rechallenged *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice were analyzed by flow cytometry for (D) peritoneal eosinophils, (E) quantitation as in (D), (F) LPMs, (G) quantitation as in (F), and (H) quantitation of the frequency of RELMα⁺ LPMs. (I) Naïve and *H. polygyrus*-rechallenged *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice were analyzed for serum anti-*H. polygyrus* IgG1 titers. (J-M) Naïve and *H. polygyrus*-rechallenged *Cd4-Cre⁺ Bhlhe40^{fl/fl}* and *Cd4-Cre⁺ Bhlhe40^{fl/fl}* mice were analyzed by flow cytometry for quantitation of (J) peritoneal eosinophils, (K) LPMs, (L) the frequency of RELMα⁺ LPMs, and (M) quantitation of SILP CD3⁺ T cells. Data are representative of or pooled from at least 3 independent experiments (A-H, J-M) or are from 2 independent experiments (I). Data are mean ± s.e.m. Significance calculated with an unpaired Student's *t*-test.



Supplemental Figure 2. BHLHE40 regulates distinct gene sets in CD4⁺ T cells from naïve and *H. polygyrus*-rechallenged mice. (A) Gene expression microarray data were analyzed for genes induced by ≥ 2 -fold in SILP CD4⁺ T cells from *H. polygyrus*-rechallenged as compared to naïve *Bhlhe40*^{+/+} mice. **(B)** Gene expression microarray data were analyzed for shared and unique *Bhlhe40*-dependent genes (≥ 2 -fold differentially expressed) in SILP CD4⁺ T cells from naïve or *H. polygyrus*-rechallenged *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice, depicted as a Venn diagram. **(C)** Gene expression microarray data were analyzed for genes differentially expressed by ≥ 2 -fold in SILP CD4⁺ T cells from naïve *Bhlhe40*^{+/+} and *Bhlhe40*^{-/-} mice. Microarray data are from 1 experiment.



Supplemental Figure 3. Bhlhe40 regulates TH2 cell cytokine production. (A) Naïve and *H. polygyrus*-rechallenged *Bhlhe40^{+/+}* mice were analyzed by flow cytometry for GM-CSF and IL-5-producing CD4⁺ T cells after *in vitro* PMA/ionomycin stimulation of SILP cells. (B) Naïve and *H. polygyrus*-rechallenged *Cd4-Cre⁺ Bhlhe40^{fl/fl}* and *Cd4-Cre⁺ Bhlhe40^{fl/fl}* mice were analyzed by flow cytometry for quantitation of the frequency of IL-4⁺, IL-5⁺, IL-13⁺, and GM-CSF⁺ CD4⁺ peritoneal T cells after *in vitro* PMA/ionomycin stimulation. (C and D) Naïve CD4⁺ T cells from *Bhlhe40^{+/+}* and *Bhlhe40^{-/-}* mice were differentiated in culture into TH2 cells and (C) restimulated to assess production of GM-CSF, IL-4, IL-5, and IL-13 by ELISA and (D) total viable cells were counted. (E) Naïve CD4⁺ T cells from *Bhlhe40^{+/+}* and *Bhlhe40^{-/-}* mice were labelled with CFSE, differentiated in culture into TH2 cells, and analyzed by flow cytometry for CFSE dilution. (F and G) Tracings of Bhlhe40 binding and vertebrate conservation at the (F) *Csf2* and (G) *Il5* loci in *Bhlhe40^{+/+}* and *Bhlhe40^{-/-}* TH1 cells (GSE113054). Data in (B-D) are pooled from at least 2 independent experiments and data in (E) is from 1 experiment. Data are mean ± s.e.m. Significance calculated with an unpaired Student's *t*-test.



Supplemental Figure 4. Further analysis of cytokine regulation of *H. polygyrus* rechallenge. (A) Naïve and *H. polygyrus*-rechallenged $Cd4\text{-Cre}^- Bhlhe40^{fl/fl}$ and $Cd4\text{-Cre}^+ Bhlhe40^{fl/fl}$ mice were analyzed by flow cytometry for quantitation of the frequency of IL-10⁺ CD4⁺ T cells after *in vitro* PMA/ionomycin stimulation of SILP cells. (B) *H. polygyrus*-rechallenged $Bhlhe40^{+/+}$, $Bhlhe40^{-/-}$, and $Bhlhe40^{-/-} Il10^{-/-}$ mice were analyzed for quantitation of *H. polygyrus* eggs/gram feces over time. (C) Naïve and *H. polygyrus*-rechallenged $Bhlhe40^{+/+}$, $Bhlhe40^{-/-}$, and $Bhlhe40^{-/-} Il10^{-/-}$ mice were analyzed for quantitation of intestinal granulomas. (D) *H. polygyrus*-rechallenged $Csf2^{+/+}$ and $Csf2^{-/-}$ mice were analyzed for quantitation of *H. polygyrus* eggs/gram feces over time. (E) Naïve and *H. polygyrus*-rechallenged $Csf2^{+/+}$ and $Csf2^{-/-}$ mice were analyzed for quantitation of intestinal granulomas. (F) Naïve and *H. polygyrus*-rechallenged $Csf2^{+/+}$ and $Csf2^{-/-}$ mice were analyzed by flow cytometry for quantitation of SILP myeloid cells. (G-I) Naïve and *H. polygyrus*-rechallenged $Csf2rb^{+/+}$ and $Csf2rb^{-/-}$ mice were analyzed by flow cytometry for quantitation of (G) peritoneal eosinophils, (H) LPMs, and (I) the frequency of RELM α^+ LPMs. Data are pooled from 2 independent experiments. Data are mean \pm s.e.m. Significance calculated with an unpaired Student's *t*-test.